EFFICACY OF A WHEY PERMEATE BASED SPORTS DRINK

By

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ABSTRACT

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Whey permeate is a by product of the cheese industry and is abundant in valuable nutrients. To our knowledge it has not been used as a base in a sports drink before. The present study was conducted to determine the effectiveness of a whey permeate based sports drink and compare it to a common commercial sports drink and to water. The whey permeate beverage was prepared in University of Wisconsin-Stout food processing lab. The whey permeate beverage was prepared by going through several processes, which include; pasteurization, fermentation, refining, centrifugation and filtration. Four Track and Field athletes volunteered to participate in this study. The athletes followed their usual practice regimen which consisted of one hour of steady running, sprinting, and jumping. Blood draws were taken in three intervals (before exercise, after exercise, and

45 minutes after consumption of one of the beverages). The blood samples were taken to a certified laboratory for assessment. The sodium concentrations (139.9-144.1 ppm) were in the expected range and the low standard error coefficient of variation indicated minor differences among the athletes. The data did not indicate any influence of the experimental beverage, water and Gatorade® on sodium concentration of blood. The potassium concentrations (3.9-4.4 ppm) were also within the expected range, but the variations were higher compared to sodium, particularly after beverage consumption. The values for calcium (9.5-10.0 ppm) and magnesium (1.8-2.0 ppm) were very similar for all three beverages. Beverage contribution to blood calcium and magnesium was not apparent. Our research did not show a significant difference among the three beverages (the experimental whey permeate beverage, water, Gatorade®) in their contribution to blood mineral levels. Surprisingly, Gatorade®, a popular sports drink, did not show mineral composition changes in blood after consumption within this particular group. Further studies will be needed to explain the effect of commercial beverages and the experimental whey permeate based sports drink.

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I wish to dedicate this thesis to all of those who supported me.

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Chapter One

Introduction

There are thousands of cheese factories that produce whey protein concentrate with concurrent generation of huge quantities of whey permeate. For example Cady Cheese Factory in Wilson, Wisconsin, produces 30,000 liters/h of permeate. Casein coagulates to form cheese curd when cheese is produced from milk. The cheese curd also entraps most of the milk fat within the protein matrix. The remaining milk components are composed of lactose, minerals and whey proteins which remain in the liquid portion referred to as whey (Choudhury, 2002). The annual production of whey in Wisconsin is approximately 21.5 billion pounds (Hugh, 2000).

Usually an ultrafiltration process can recover the proteins in whey by separating molecules according to their size. These recovered proteins are dried and marketed as whey protein concentrates (WPC) for use as ingredients in various food products. The liquid that is passed through the membrane is known as whey permeate and is high in lactose and minerals (Choudhury, 2002). Cady Cheese Factory disposes this liquid portion of whey by spraying it on nearby farms. This method of disposal is a loss of valuable nutrients and is less than desirable (Choudhury, 2002).

Disposal of whey permeate is challenging to many cheese producing plants across the United States. Many manufacturers have different methods of handling this disposal problem. Common disposal methods include; spreading the whey permeate on land as a fertilizer, feeding it to farm animals, and extracting lactose through reverse osmosis and crystallization to reduce its BOD (Biological Oxygen Demand) before disposing the liquid into the public sewer system. Some of the methods of disposal are harmful to the environment, while others are costly. Disposal and utilization of whey permeate is still a problem facing the dairy industry (Choudhury, 2002).

Attempts have been made to produce beverages from whey and whey permeate (Gillies, 1974; Girsh, 1999). These attempts have been unsuccessful because of an unacceptable cheesy odor, color, and aftertaste in the final product. Preliminary studies in our lab indicated that lactose could be reduced or eliminated and the unacceptable cheesy odor and color would no longer be present. Through several processes (pg. 33 - 35) a clear liquid is obtained which is high in minerals and therefore could be used as a base for a sports drink.

Fluid loss during exercise is the body's primary mechanism for eliminating excess heat. Evaporation of water cools the skin, blood, and decreases the temperature of the inner core. This causes blood volume to decrease, increasing the demand on the cardiovascular system. The rate of fluid loss from sweat during exercise varies from 0.9 – 1.9 L/h. Thirst does not occur until there is a 1% loss in body weight. When fluid losses reach 2 – 4% of total body weight, physical performance is impaired. Minerals are lost with water during sweating. Food industries have developed numerous sports drinks that are commercially available in the market to compensate for the loss of water and electrolytes. Properly formulated sports drinks encourage voluntary fluid consumption, stimulate rapid fluid absorption, provide carbohydrate for improved performance, augment physiological response, and increase rehydration (Murray & Stofan, 2001). Whey permeate contain large quantities of minerals that are needed to formulate a sports drink. In addition to sodium and potassium, whey also contains other important minerals such as calcium and magnesium.

Statement of The Problem

The purpose of this study was to determine the effectiveness of a whey permeate based sports drink when compared to a popular commercial sports drink and water.

The specific objectives of this research are as follows:

1. Formulate the sports beverage to meet desirable needs (6% carbohydrate solution, 110 mg sodium and 30 mg potassium per 240 mL serving).
2. Compare the effects on rehydration in athletes of the whey permeate based sports drink to a commercial sports drink and water.

Chapter Two

Review of Literature

Fluid Needs During Exercise

Water is the largest component of the body, representing approximately 50% of body weight for the adult female and 60% of body weight for the adult male. Total body fluid is distributed between the extracellular fluid compartment and the intracellular compartment. The extracellular fluid is composed of all the fluid that is outside the cells and is further divided into the interstitial fluid and the noncellular part of the blood called the plasma (intravascular fluid). A separate compartment of fluid called transcellular fluid is only about 2% of total body fluid. Average electrolyte concentrations in sweat, plasma, and skeletal muscle are shown in Table 1 (Driskell & Wolinsky, 1999).

Table 1

Comparison average electrolyte concentrations in sweat, plasma, and skeletal muscle

Variable	Na+ (meq/L)	Cl- (meq/L)	K+ (meq/L)	Osmolarity (mosmol/L)
Sweat	40 to 60	30 to 50	1 to 5	80 to 150
Plasma	140	101	5	290
Muscle	9	6	162	290

Adapted from Driskell & Wolinsky *Macroelements, Water, and Electrolytes.* (1999).

Water serves as a transport mechanism and reactive medium. Gases diffuse across surfaces moistened with water. Nutrients and gases require water to travel throughout the body. Waste products are excreted through water in urine and feces. Water with other proteins lubricates joints and protects organs such as the heart, lungs, intestines, and eyes. Water provides structure and form to the body through the turgor provided for body tissues. Water is also used to control body temperature. Water can stabilize body heat by absorbing considerable heat with only small changes in total body temperature (McArdle & Katch, 1999).

Individuals have different fluid needs based on physical activity. Many resources indicate that a sedentary adult require 2 L (8 cups of fluid) per day. In physically active individuals daily fluid needs are above 2 L of fluid. Some individuals, including athletes and workers may require as much as 10 L of fluid. Fluid loss also occurs through urine excretion, gastrointestinal tract through feces, exocrine sweat glands, and through the respiratory tract and the skin (Rosenbloom, 2000). Environmental conditions, individual metabolic rate and the volume of fluid excreted also play an important role in fluid needs (Rosenbloom, 2000). Fluid requirements increase secondary to sweat loss during physical activity. Fluid needs can be met from a variety of sources including milk, soft drinks, fruit juices, sports drinks, soup, and other fluids (Rosenbloom, 2000).

Urine output is actually lower in athletes than sedentary individuals. Physical activity tends to reduce urine production as the kidneys conserve water and sodium to compensate for losses due to sweating. Warm weather triggers the body to conserve fluid. Fluid losses add stress to the body's regulatory system, which causes thirst to be an inadequate stimulus for fluid intake, which in return causes dehydration (Rosenbloom, 2000). Table 2 illustrates daily fluid losses for an average 70 kg athlete.

Table 2

Daily fluid loss (in mL) for a 70 kg athlete

	68°F (mL)	85°F (mL)	Exercise in 85°F (mL)
Skin	350	350	350
Respiratory	350	250	650
Urine	1,400	1,200	500
Feces	100	100	100
Sweat	100	1,400	5,000
Total Losses	2300	3300	6,600

Adapted from: Guyton. *Textbook of Medical Physiology*. 8th ed. (1991).

Calcium and Magnesium

Calcium

Ninety nine percent of calcium is found in the bone and teeth, whereas the remaining 1% is distributed amongst the extracelllular fluids, intracellular structures, cell membranes and several soft tissues (Rosenbloom, 2000).

Functions of calcium include the following:

- Bone metabolism
- Blood coagulation
- Nueromuscular excitability
- Cellular adhesiveness
- Transmission of nerve impulses

- Maintenance and function of cell membranes
- Activation of enzyme reactions and hormone secretions.

Serum calcium level is maintained within a range of 2.2 to 2.5 mmol / L by the parathyroid hormone (PTH), vitamin D, and calcitonin. PTH responds when serum calcium levels decrease below the normal range. PTH increases calcitriol synthesis in the kidney to regulate the serum calcium level.

Individuals who are physically active are affected by low calcium intake because some calcium is lost in sweat and urine. Individuals who exercise in heat have higher calcium losses in sweat (Rosenbloom, 2000). Unfortunately critical roles of calcium and other minerals (not including sodium and potassium) have not been identified for use in sports drinks (Maughan & Murray, 2001).

Magnesium

Bones contain approximately 60 to 65% of total body magnesium whereas 27% is present in muscle, 6 to 7% is found in cells and 1% found in extracellular fluid (Rosenbloom, 2000). Magnesium is an important factor when it comes to exercise. Magnesium is responsible for many metabolic processes required for exercise. For example, functions include; mitochondrial function, carbohydrate synthesis, lipid and protein synthesis, energy delivering processes, and coordination of neuromuscular activity (Rosenbloom, 2000). Excretion of magnesium in urine and sweat is increased in individuals who exercise. Individuals who have low magnesium levels could suffer from muscle spasms during exercise (Rosenbloom, 2000). Normal calcium and magnesium concentrations in sweat, plasma, and intracellular water are shown in Table 3.

Table 3

Concentration of calcium and magnesium found in sweat, plasma, and intracellular water

	Sweat (mmol/L)	Plasma (mmol/L)	Intracellular (mmol/L)
Calcium	0 – 1	2.1 – 2.9	0
Magnesium	<0.2	0.7 – 1.6	15

Adapted from Maughan and Murray; Sports Drinks, 2001

Plasma Variables

Loss of electrolytes occurs in urine and sweat. Active individuals who produce sweat on a daily basis can lose large amounts of electrolytes, including sodium (20 to 100 + mmol/L) chloride, and potassium (usually <10 mmol/L). Individuals lose different amounts of sodium through sweat. This explains why some individuals develop large sodium deficits compared to other individuals. Sodium chloride losses in sweat have increased the risk of heat-related problems and muscle cramps (Rosenbloom, 2000).

Sodium and Potassium

Sodium and potassium (electrolytes) are dissolved electrically charged particles called ions found in blood plasma and extracellular fluid. Electrolytes regulate the fluid exchange within various fluid compartments within the human body. Potassium is the most important intracellular mineral (McArdle & Katch,1999). Dehydration affects electrolyte blood plasma levels. Therefore plasma variables are often measured to determine electrolyte changes secondary to dehydration. As dehydration occurs electrolytes are measured falsely high because of the increase in concentration versus fluid. Table 4 represents concentrations of electrolytes in blood serum, sweat and carbohydrate-electrolyte concentrations of some common beverages.

Table 4

Blood serum, sweat, and carbohydrate concentrations of some common beverages

	Na+ MEq/L	K+ Meq/L	Ca++ Meq/L	Mg++ Meq/L	Cl- Meq/L	Osmolality MOsm/L	Carbohydrate g/ L
Blood Serum	140	4.5	2.5	1.5-2.1	110	300	------
Sweat	60-80	4.5	1.5	3.3	40-90	170-220	-------
Coca-Cola®	3.0	------	--------	-------	1.0	650	107
Gatorade®	23.0	3.0	-------	-------	14.0	280	62
Fruit Juice	0.5	58.0	--------	-------	------	690	118
Pepsi-Cola®	1.7	trace	-------	------	Trace	568	81
Water	trace	trace	--------	--------	Trace	10-20	----------

Adapted from: McArdle, Katch, & Katch, 1999.

Magnesium

Magnesium is responsible for playing a role in maintaining the nervous, musculoskeletal, and cardiovascular systems. However hypomagnesemia is most likely the underdiagnosed electrolyte deficiency. There are many factors, which may alter both urinary and serum Mg. This includes the following; acid base balance, mental stress, physical stress, and volume expansion. Serum Mg is not a routine screen and can also mimic other electrolyte imbalances. Serum Mg may be a limited prognostic value, because only about 1% of total body serum is present in the extracellular fluid compartment (Driskell & Wolinsky, 1999). Mg is not a valid measurement in relation to hydration status, however for the purposes of this study a serum Mg level was taken to see the effects of the whey permeate based beverage which contains a small amount of Mg naturally present in the whey permeate.

Calcium

The average adult human body contains approximately 1 kg of calcium, which 99% is found in the skeleton in solid form. Calcium found in the tissues is bound to various organic or inorganic molecules or as an ion in a solution. Blood calcium is found nearly entirely in the plasma, where approximately half is found bound with proteins, for example albumin. The remainder is completely in ionic form and a small amount (<7% is found as citrate, phosphate, or various complexes) (Driskell & Wolinsky, 1999). Plasma calcium is not a good indicator or reflection of dehydration. However in relation to this study a serum plasma calcium level was taken to see the effects of the whey permeate based sports drink, which contains calcium naturally present in the whey permeate.

Current Studies Regarding the Use of Sports Drinks by Athletes

Gonzale-Alonso et. al. (1992) assessed the effectiveness of two common rehydration beverages: a caffeinated diet cola, and a 6% carbohydrate–electrolyte solution. Two beverages were compared with water regarding whole body rehydration, gastric emptying, and blood volume. It was determined that a caffeinated beverage was less effective than water for whole body rehydration, while a carbohydrate electrolyte beverage appeared to be more effective than water or a caffeinated beverage (Gonzale –Alonso, et. al, 1992). Other researchers from the University of Quebec found that fructose containing beverages are not as readily available for energy as glucose containing beverages (Massicotte, 1989).

Mitchell (1988) studied the effects of ingesting water versus carbohydrate containing beverages (5%, 6%, and 7.5% carbohydrate solutions, respectively) on gastric emptying and performance. It was determined that exercise performance was improved with the ingestion of the carbohydrate containing beverages. Mitchell found that the gastric emptying rates for the carbohydrate containing beverages were similar to the water placebo. It was concluded that consuming carbohydrate beverages could enhance performance (Mitchell, 1988). Coyle (1986) found that carbohydrate ingestion during prolonged exercise allowed participants to exercise an additional hour compared to the water placebo. Coyle (1986) attributed the ergogenic effect of the carbohydrate consumption to an increase in blood glucose level and an increase in carbohydrate oxidation by working muscles. These findings demonstrated the importance of maintaining blood glucose levels during exercise by consuming carbohydrates.

Gisolfi (1995) studied the effect of a 6% carbohydrate electrolyte solution containing different sodium concentrations (0, 25, 50 mEq/L of sodium) on absorption of fluid in the small intestine using a segmental perfusion technique. Gisolfi (1995) found that the level of carbohydrate in a solution is a more important factor in determining intestinal fluid absorption than sodium (Gisolfi, 1995). Cade et. al (1972) determined that serum sodium levels were significantly higher when athletes consumed nothing and significantly less when a glucose-electrolyte solution was consumed. Murray (1987) discovered that a beverage containing electrolytes will help to maintain fluid and electrolyte balance during exercise. Murray states that a beverage should be designed to enhance oral intake, maintain endogenous carbohydrate stores and enhance performance.

Dehydration

Dehydration is considered to be an imbalance in fluid dynamics and occurs when fluid intake does not replenish fluid loss. Moderate exercise usually produces 0.5 to 1.5 liters sweat over a one-hour period. Significant fluid loss occurs during several hours of heavy exercise in a hot environment. Water loss also occurs in power athletes such as wrestlers, boxers, weight lifters and rowers.

Intracellular and extracellular compartments contribute to dehydration, which can rapidly reach levels that impede heat dissipation, reduce heat tolerance, and severely compromise cardiovascular function and exercise capacity. Risks of heat illness increases significantly when an individual exercises in a dehydrated state. Plasma osmolality increases when sweating occurs because sweat is hypotonic with other body fluids. The hypovolemia caused by sweating increases the osmolality of blood plasma (McArdle, Katch, & Katch, 1999).

Dehydration impairs physiologic function and thermoregulation. As the progression of dehydration occurs and plasma volume decreases, peripheral blood flow and sweating rate diminish and thermoregulation becomes progressively more difficult. This contributes to an increased heart rate, perception of effort, core temperature, and premature fatigue than under normal hydration. (McArdle, Katch, & Katch, 1999).
For example, a fluid loss of 1% of body mass increases rectal temperature compared the same exercise performed when fully hydrated. For men and women, a pre-exercised dehydration equivalent to 5% of body mass significantly increases rectal temperature and heart rate, while decreasing the rate of sweat production. (McArdle, Katch, & Katch, 1999).

Blood plasma supplies the majority of water lost through sweating, which explains that progressive sweat loss adversely impacts maintenance of cardiac output. The reduction in cardiac output increases systemic vascular resistance and reduces blood flow to the skin. A reduction in cutaneous blood creates a major avenue for heat dissipation. When dehydration occurs, circulation and temperature regulation decreases capacity to meet both metabolic and thermal demands of exercise (McArdle, Katch, & Katch, 1999).

When a person is at rest they lose 450 to 600 mL of water from the skin and lungs, this represents an insensible heat loss of 12 to 16 kcal per hour. When body temperatures begin to rise, evaporation of diffused water cannot be adjusted. Instead the rate of heat loss by evaporation increases. In slightly warmer weather, copious amounts of water are lost by sweating, so much that individuals who make an effort to maintain euhydration are not completely successful. Voluntary intake of fluids rarely replaces more than about half the water that is lost by sweating. This leads to “involuntary” dehydration. Unfortunately most individuals do not rehydrate voluntarily even when water is available, leading to “voluntary” dehydration (Driskell & Wolinsky, 1999).

Losing 9 to 12% of body water can be fatal. Individuals who exercise for more than 30 minutes without consuming water would still add water to their total body water by body compensation. First of all as exercise intensity increases metabolism increases which produces approximately 100 to 150 mL of water per hour. Next muscle glycogenolysis liberates about 3 to 4 grams of water per gram of glycogen. This increases total body water by 600 to 650 mL per hour. However this is not enough to maintain a non-dehydration status (Driskell & Wolinsky, 1999).

Physiological Determinants of Fluid Intake

Many physiological influences on thirst have been detected. Much of the research conducted have not specifically investigated causal relationships between physiological substrates and fluid intake, however many researchers have focused on thirst. Thirst is an important dependent measure in the understanding of fluid intake. Physiological conditions influence thirst, or the “desire to drink” which is viewed as a direct influence on drinking behavior (Maughan & Murray, 2001).

Two principal physiological causes of thirst and drinking are cellular dehydration and hypovolemia (extracellular dehydration). Cellular dehydration is the most important physiological cause. A small decrease in cellular hydration status will result in thirst, where even large reductions in plasma volume will not result in thirst. Approximately 64 to 85% of drinking following water deprivation is due to cellular dehydration, and that only about 5 to 27% of drinking is accounted by hypovolemia (Maughan & Murray, 2001).

Rehydration

Replacing fluid appropriately maintains the exceptional potential for evaporative cooling of acclimatized humans (McArdle, Katch, & Katch, 1999). Glycogen depletion during exercise impairs performance for endurance athletes, but does not increase the risk for health and safety. However, inadequate fluid replacement impairs exercise capacity and creates life-threatening disturbances in fluid balance and body core temperature. (McArdle, Katch, & Katch, 1999).

Body weight changes illustrate the amount of fluid that is lost during exercise. Changes in body weight can be used as a tool to indicate the adequacy of rehydration during and after exercise. Urine color is also an appropriate measure of dehydration. Voiding small volumes of dark yellow urine with a strong odor indicates inadequate hydration. Individuals who are well hydrated produce large volumes of light colored urine without a strong odor (McArdle, Katch, & Katch, 1999).

Please see Table 5 for fluid recommendations during a 90-minute exercise interval.

Table 5

Fluid recommendations during a 90 – minute exercise interval

Weight Loss LB kg		Minutes between water breaks	Fluid per break Oz mL	
8	3.6	Recommend	-	-
7.5	3.4	No practice	-	-
7	3.2	10	8-10	266
6.5	3.0	10	8-9	251
6	2.7	10	8-9	251
5.5	2.5	15	10-12	325
5	2.3	15	10-11	311
4.5	2.1	15	9-10	281
4	1.8	15	8-9	251
3.5	1.6	20	10-11	311
3	1.4	20	9-10	281
2.5	1.1	20	7-8	222
2	0.9	30	8	237
1.5	0.7	30	6	177
1	0.5	45	6	177
0.5	0.2	60	6	177

Adapted from McArdle, Katch, & Katch, 1999

This table demonstrates the amount of fluid to consume when a particular weight loss is present. For example water breaks are recommended for a weight loss of 0.2 to 3.2 kg during a 90 minute exercise interval (10 to 60 minutes between water breaks depending on the amount of weight lost). If an individual reaches as much as 3.4 to 3.6 kg body weight loss, exercise should be stopped immediately.

Consumption of palatable flavored beverages regulates voluntary rehydration. (Maughan & Murray, 2001). A small amount of sodium added to beverages facilitates an increase in complete rehydration compared to plain water (McArdle, Katch, & Katch, 1999).

In addition to sodium adding a small amount of potassium (2 to 5 mmol / L) may increase the water retention in the intracellular space, and may decrease any extra potassium loss from sodium retention by the kidneys (Schedl, 1994). Kidneys form urine continuously which explains why ingested fluid after exercise must be larger (25 to 50%) than sweat loss during exercise to restore fluid balance. Unfortunately, without a sufficient sodium content, excess fluid only increases urine output without any benefit to rehydration (Shireffs, 1996).

Sports Drinks

According to Maughan and Murray (2001) sports drinks obtain public visibility because of their link with exercise and competitive sports. Sports drink sales in 1998 reached $2.2 billion dollars. Sports drink formulation comes from science. The basic science that underlies the effectiveness of sports drinks stems from the development of oral rehydration solutions for the treatment of diarrhea. Sports drinks are also referred to as carbohydrate-electrolyte beverages, electrolyte replacement drinks, or isotonic drinks. The formulation specifications for beverages are generally composed of monosaccharides, disaccharides, and possibly maltodextrins, in concentrations averaging from 6% to 9% weight per volume. Sports drinks usually contain small amounts of electrolytes such as sodium, potassium, chloride, and phosphate and usually come in fruit related flavors (Maughan & Murray, 2001). Table 6 illustrates the composition of selected sports drinks and common beverages.

Table 6

Sodium, potassium, and carbohydrate concentration of some commercial and whey permeate experimental beverages

Beverage	Manufacturing Company	Sodium (mmol/L)	Potassium (mmol/L)	Carbohydrate (% wt/vol)
AllSport®	Pepsico	10	5	8-9
Gatorade®	Quaker Oats Co.	20	3	6
Powerade®	(Coca - Cola)	5	3	8
Whey permeate based sports drink	Experimental	20	3	6

Adapted from Maughan & Murray, 2001

A properly formulated sports drinks should deliver the following benefits depending on certain circumstances: (Maughan & Murray, 2001)

- Encourages voluntary consumption of fluid
- Stimulates rapid absorption of fluid
- Supplies carbohydrate for performance improvement
- Encourages physiologic response
- Provides fast rehydration

Beverage osmolality (i.e. number of particles in solution) plays a role on gastric emptying. Increasing the amount of carbohydrates or electrolytes in a sports drink causes an increase in the osmolality of the beverage which could slow gastric emptying. Research indicates that sports drinks should be isotonic or hypotonic to ensure rapid gastric emptying (Maughan & Murray, 2001).

Sodium chloride affects flavor and functional properties of a sports drink. According to Maughan and Murray (2001) sodium chloride is added to sports drinks to:

- "Smooth" flavor profiles
- Stimulate fluid consumption by maintaining the osmotic and volume dependent stimuli for drinking
- Ensure ample sodium concentration in the intestinal lumen
- Provide osmotic impetus for extracellular fluid volume maintenance
- Encourage adequate drinking and rehydration following physical activity

Studies have shown that sodium ingestion during exercise is critical for plasma sodium level maintenance and to help prolong duration of exercise (Maughan & Murray, 2001).

Carbohydrate concentration of a sports drink should be at a 6% to 7% (weight/volume). Levels beyond this have been shown to significantly decrease the rate of fluid absorption by decreasing gastric emptying and increasing the risks of gastrointestinal discomfort at no benefit to performance (Maughan and Murray, 2001).

Sports drinks are one of the best-researched food products in the world. Small changes in product formulation (i.e. high carbohydrate concentration, low sodium concentration) can alter the physiological and performance responses to ingesting sports drinks during exercise (Maughan & Murray, 2001).

Whey Permeate

When milk is used to produce cheese, coagulation occurs from casein to form cheese curd. This cheese curd contains the milk fat within the matrix of the protein. The remaining milk components such as lactose, minerals, and whey proteins remain in a liquid portion, which is referred to as whey. Whey permeate is the liquid portion that passes through the membrane. Whey permeate is a valuable in potassium, magnesium, calcium, and sodium. At this time, many cheese factories use the whey permeate as a fertilizer by spreading it on their land, however this method is harmful to the environment because of its high BOD (Biological Oxygen Demand) (Choudhury, 2002).

Other methods that have been used have been costly. This includes extracting the lactose through reverse osmosis and crystallization to reduce its BOD before disposing the liquid into the public sewer system (Choudhury, 2002).

Unfortunately attempts that have been made to produce drinks from the whey permeate have failed because of the cheesy odor and smell in the whey permeate (Gillies, 1974; Girsh, 1999).

It has been determined that whey permeate can be modified to remove or reduce lactose and eliminate the unacceptable cheesy color and odor. The final product is clear and is rich in minerals and can be used as a base for sports drinks and other beverages.

Chapter Three

Materials and Methods

Cheese whey permeate was donated by Cady Cheese Factory Inc. (Wilson, Wisconsin). Rhodia Food (Madison, Wisconsin) donated the starter culture used to eliminate the lactose (naturally present in the cheese whey permeate), Lactobacillus Helvitetcus (LH 100). Ammonium hydroxide (NH_4OH) was purchased through Spectrum Chemical Mfg. (New Brunswick NJ) to regulate the pH of the whey permeate during the fermentation stage. Morton International (Chicago, IL) donated sodium chloride, (Culinox 999) which was added to the final product to reach optimum (110 mg/240 mL) sodium concentration in the sports beverage. Dextrose (Gallipot Anhydrate Dextrose) was purchased through Woodwinds pharmacy. Orange flavoring (Watkins) and food coloring (Schilling) was purchased through a Watkins dealer and at a local grocery store, respectively.

Preparation of the Cheese Whey Permeate Base

I. *Pasteurization*. The fresh cheese whey permeate was stored at 4°C for approximately 1 hour before use. The fresh whey permeate was next measured equally into six sterile plastic bottles with lids (650 mL whey permeate in each container). The containers were placed in a hot water bath and pasteurized at 80°C for five minutes to destroy the microorganisms used during cheese making. The containers (containing the cheese whey permeate) were next cooled to 10°C in a cool water bath.

II. Fermentation.

Lactobacillus Helvitecus (LH 100) was added to the whey permeate (0.5 g per 100 mL). This stage is aimed at removing lactose by fermenting it into lactic acid. The whey permeate was next placed in a water bath (Sheldon Manufacturing, VWR Scientific Products, model # 1217) at 41.5°C and 70 rpm. The pH meter (IQ Scientific Instruments, model # IQ240) was used to determine the pH of the cheese whey permeate. (pH 4.01 and pH 7.0 Thermo Orion Application Solution, Beverly MA buffers were used to calibrate the pH meter). The pH was checked every 30 minutes for the first four hours and than every hour thereafter for a total of 48 hours. Food grade ammonium hydroxide (NH_4OH) was added until the pH reached approximately 6.0 (0.5 mL per 650 mL at a time) was added to the whey permeate when the pH dropped below 5.0. After 48 hours the pH of the whey permeate was stabilized at 5.7. The fermentation stage was terminated by pasteurizing the product at 80.0°C for two minutes.

II. Refining

Color and off flavor components in the fermented cheese whey permeate were removed by adsorption. Activated carbon (0.008 g/L) was added to the product to initiate this process.

III. Filtration/Centrifugation

The product with the activated carbon was placed into centrifuge bottles and centrifuged (Beckman Coulter Allegra 6KR) for 20 minutes at 4000 rpm. This liquid portion was than filtered until a complete clear liquid remained with no reminants. The product was again pasteurized at 80°C for five minutes. The process for production of a sports drink base from cheese whey permeate is summarized in Figure 1.

Figure 1. Process for production of a sports drink from cheese whey permeate.

Cheese Whey Permeate

⇓

Pasteurization

⇓

Fermentation

⇓

Refining

⇓

Filtration/Centrifugation

Lactose, galactose, and glucose determination in whey permeate by high performance liquid chromatography (HPLC) Hewlett Packard 1040A HPLC detection system)

Standards of lactose, galactose, and glucose were prepared containing 0.5, 1.0, and 2.0% by weight by dissolving appropriate quantities of each sugar in 100 mL of distilled water. After all standards were prepared, they were mixed thoroughly. A syringe with a membrane filter attached was used to fill the HPLC sample bottles. Whey permeate was prepared for HPLC analysis. Ten fold dilutions were prepared from the whey permeate. A 10 mL sample of whey permeate was pipetted into a 100 mL volumetric flask, and then filled with distilled (MilliQ) water up to the mark on the neck of the flask. A small amount of sample was transferred into a syringe fitted with a membrane filters. One at a time the samples were placed into a labeled vial.

The HPLC was set for the following conditions:

Mobile phase: 75 % acetonitrile / 25% MilliQ water (v/v)

Flow rate: 1.5 mL / min

Refractive index detector attenuation / 8

Injection volume: 30μL

Run time: 10 min

One by one each sample was injected into the HPLC. After a sample was taken it passed through the guard cartridge, which filtered out any substances that would harm the column. From the guard cartridge the sample immediately proceeded into the column. The lactose, galactose, and glucose was separated and was measured by the differential reflectometer. The reflectometer signaled the computer to record the information in peaks on a graph. These peaks were graphed according to surface area to indicate the amount of each sugar present in the cheese whey permeate.

Determination of Sodium, Calcium, Magnesium, and Potassium in Whey Permeate by Atomic Absorption Spectrophotometry (Solaar S series, 9499, 400, 300001, 270302, Cambridge Kingdom)

Standards were prepared using sodium, potassium, lanthanum, magnesium, and calcium in 0.10, 0.25. 0.50, 0.75, 1.0, 2.5, and 5.0 mg / L respectively.

All chemicals were analytical grade. In preparation of Ca stock, 50 mL deionized water was added into $CaCO3$ powder at first. Concentrated HCL was then added dropwise to completely dissolve the solid. Deionized water was used to dilute to 1 L. In preparation of Mg stock, 50 mL deionized water was added into Mg strip at first.

Approximately 10 mL concentrated HCL was then added gradually to completely dissolve the metal. Deionized water was used to dilute to 1 L. Samples of the whey permeate were prepared using the following concentration: 1:1000 (1 mL whey permeate diluted to 1,000 mL) Two identical samples were used after trial and error using different concentrations.

Settings for Atomic Absorption Spectrophotometry

Potassium

Wavelength – 766.5 nm

Measurement time – 4.0 seconds

Signal type – Continuous

Flame type – air C_2H_2

Sodium

Wavelength – 285.2 nm

Measurement time – 4.0 seconds

Signal type - Continuous

Flame type – air C_2H_2

Dilution of whey permeate and addition of sodium, dextrose, flavoring and coloring

The cheese whey permeate was diluted with pure water (same water used as a beverage in the study, Member's Mark Natural Spring Water, sodium and mineral free) 1:21 before flavoring and coloring was added. Sodium chloride (Cullinox 999) was added to the whey permeate to reach a desired level of 110 mg sodium per 240 mL serving of sports drink. Orange flavor (Watkins) and color (Schilling) was later added to the final product.

Determination of Electrolyte Composition in Athletes

The effectiveness of three beverages was compared for promoting rehydration. The beverages consisted of the whey permeate based sports drink, a commercial sports drink, and pure water (Member's Mark Natural Spring Water, sodium and mineral free) with no additives.

Four UW-Stout athletes (Ages 19-20) volunteered to participate in this study. The athletes were weighed and a forearm blood sample was taken by a certified lab technician prior to their usual practice regimen. They were not asked to change their usual practice schedule in any way. After completion of their practice, which included 60 minutes of continuous running, sprinting, and jumping the athletes were re-weighed and another blood sample was taken. Each athlete was given three cups of a sports beverage per pound of body weight lost. Another blood sample was taken from the athletes after approximately 45 minutes of rest. The blood samples were taken to a Myrtle Werth Hospital certified lab and analyzed for electrolyte composition. Two trials were done for each beverage. The results of the blood tests were compared and statistically analyzed (Anova, etc.).

Analysis of Blood Composition (Completed at Myrtle Werth Hospital Certified Lab)

Sodium and Potassium

Sodium and potassium composition in the blood were detected using potentiometry. A slide, consiting of a dry, multilayered, analytical element that uses potentiometry for measurement of sodium or potassium ions was used. The slide consists of two ion selective electrodes, each containing methyl monsin (an ionophore for sodium), or valinomyacin (an ionophore for potassium) a reference layer, and a silver and silver chloride layer coated on a polyester support. Next 10 μL of blood and 10μL of the electrolyte reference fluid were applied to separate halves of the slide, both fluids migrated towards the center of the paper bridge. A stable liquid junction was formed connecting the reference electrode to the sample indicator electrode. Each electrode produces an electrical potential in response to the activity of sodium or potassium applied to it. The potential difference poised between the two electrodes is proportional to the sodium or potassium concentrations in the sample.

Calcium

Calcium concentration in the blood was measured using spectrophotometry. A calcium slide is a multilayered, analytical slide. Next 10 μL of blood is distributed on the spreading layer, from there it is distributed equally to the underlying layers. The bound calcium is dissociated from binding proteins, allowing calcium to penetrate through the spreading layer to the underneath reagent layer. There the calcium forms a complex with Arsenazo III dye causing a shift in the absorption maximum. After the incubation period the relection density of the colored complex is measured spectrophotometrically. The amount of colored complex formed is proportional to the calcium concentration in the sample.

Magnesium

Magnesium in the blood sample was determined by reflection density. To begin 10 μL of blood was distributed on the spreading layer from there, the sample was distributed equally to the underlying layers. Magnesium both free and protein bound from the sample reacts with the Formazan dye, a derivative of the reagent layer, a high magnesium affinity of the dye associates magnesium from the binding protein. The resulting magnesium dye complex causes a shift in the dye absorption maximum from 540 nm to 630 nm. The amount of dye complex formed is proportional to the magnesium concentration present in the sample.

Chapter Four

Results and Discussion

This study evaluated the effectiveness of a sports beverage made from a cheese whey permeate base. To our knowledge, a whey permeate sports beverage has not been used in this type of application before. The performance of the beverage was compared with water as well as with Gatorade®, a popular commercial sports beverage.

Processing of Whey Permeate

The sodium and potassium concentrations of whey permeate before and after processing (a 4-step process) are presented in Table 7. There was a slight decrease in sodium (1.6%) concentration following processing. However, potassium loss (8.5%) was higher than sodium. Potassium could possibly be retained by starter culture or activated charcoals, used during processing of cheese whey permeate. This study focused on evaluation of liquid whey permeate after processing, and therefore no attention was paid on analysis of solid residues at each stage of processing. Future development of this beverage should analyze partitioning of minerals in different phases at each stage of processing.

Table 7

Sodium and potassium composition of the whey permeate base before and after processing

Mineral	Before Processing (mg/L)	CV (%)	After Processing (mg/L)	CV (%)	% Loss
Na	431.9±1.6	0.29	424.8±1.3	0.25	1.6
K	2967.0±7.6	0.25	2715.6±5.3	0.20	8.5

The pH of the whey permeate stabilized at 5.7 after 48 hours of fermentation by *Lactobacillus helveticus* (LH 100). This was done to ensure production of a lactose free beverage. The HPLC analysis of processed whey permeate indicated absence of lactose and presence of small amounts of glucose (1.33 ppm) and galactose (0.23 ppm). However, in commercial practice such long fermentation time (48 hours) may not be desirable. Other alternatives for faster elimination of lactose should be evaluated.

The color and odor of whey permeate changed drastically during and after processing. Fresh whey permeate had a transparent lime green color and a cheesy odor. After fermentation, the permeate became milky with the cheesy odor persisting. Treatment with activated charcoal removed both the milky color and odor. Removal of food grade activated charcoal by centrifugation resulted in a colorless and odorless whey permeate base.

Comparison of the effects on Rehydration in Athletes

The sodium and potassium levels of blood before and following exercise and 45 minutes after consumption of the beverages are presented in Table 8. The sodium concentrations (139.9-144.1 ppm) were in expected range and the low coefficient of variation indicated minor differences among the athletes. The data did not indicate any influence of the experimental beverage, water and Gatorade®, on sodium concentration of blood. The potassium concentrations (3.9-4.4 ppm) were also within the expected range, but the variations were higher compared to sodium, particularly after beverage consumption as indicated by high coefficient of variation. This high variation may be due to differences in potassium transport among the athletes.

Table 8

Sodium and potassium concentration of blood before exercise, following exercise, and after consumption of the beverages

Mineral	Time of Blood Sampling	Gatorade®		Water		Whey permeate beverage	
		Mean±SE	CV (%)	Mean±SE	CV (%)	Mean±SE	CV (%)
Na	Before Exercise	141.0±1.1	1.5	141.6±0.8	1.2	140.3±1.6	2.2
	Following exercise	143.0±0.9	1.3	144.1±1.2	1.6	141.9±1.4	2.0
	After beverage consumption	140.5±1.1	1.5	141.0±1.0	1.4	139.9±1.5	2.1
K	Before exercise	4.4±0.1	2.5	4.4±0.2	4.5	4.2±0.3	2.1
	Following exercise	4.0±0.1	3.5	4.1±0.1	6.6	3.9±0.5	3.8
	After beverage consumption	4.2±0.3	7.0	4.2±.0.2	9.6	3.93±0.2	9.4

The analysis of variance (Table 9) corroborated above findings and showed no significant differences in blood sodium and potassium levels at any blood sampling period.

Table 9

Analysis of variance (ANOVA) of data of on the mineral profile (Na and K) of blood before and following exercise and after consumption of beverage

Mineral	Source of Variation	DF	Gatorade®		Water		Whey Permeate beverage	
			MSS	F	MSS	F	MSS	F
Na	Time of blood sampling	2	7.000	1.68	10.900	2.670	4.520	0.517
	Error	9	4.170		4.100		8.740	
K	Time of blood sampling	2	0.100	2.64	0.138	1.570	0.092	1.100
	Error	9	0.038		0.088		0.084	

Analysis of variance (Table 10) also indicated no significant difference among the beverages in influencing sodium and potassium concentration of blood during rehydration following exercise.

Table 10

Analysis of variance (ANOVA) of data of on the mineral profile (Na and K) of blood after consumption of Gatorade®, water and whey permeate beverage

Source of Variation	DF	Na		K	
		MSS	F	MSS	F
Type of beverage	2	1.27	0.221	0.068	0.534
Error	9	5.74		0.126	

Carter and Gisolfi (1989) examined the affects of a carbohydrate electrolyte (CE) beverage following a 3 hour cycling exercise in a controlled hot environment (Temperature = 31.5±0.7°C and RH = 22.3±2.7%). When CE beverage and water were consumed, higher levels of blood sodium and potassium were observed with CE beverage. Another study investigating rehydration after exercise observed that individuals who consumed a carbohydrate electrolyte beverage instead of water were more hydrated (higher levels of Na and K) following a 2 hour resting period (Gonzalez–Alonso, et al. 1992). We did not observe any such differences due to consumption of the experimental whey permeate beverage, water and Gatorade®. However, our experimental conditions were much different than those employed by Carter and Gisolfi (1989) and Gonzalez–Alonso, et al. (1992). A change in our experimental conditions (longer exercise period, longer resting period, controlled environment, more volunteers, standardized diet, beverage consumption during exercise) would be expected to yield different results and may prove the benefit of consuming whey permeate beverage for restoring hydration status.

Other Benefits of the Experimental Whey Permeate Beverage

Calcium and magnesium are naturally present in whey permeate beverage. Other commercial sports drinks do not have these essential minerals. The calcium and magnesium concentrations of whey permeate base are 800 mg/L and 89 mg/L, respectively. The sports beverage prepared from the base contains 38 mg/L calcium and 4.2 mg/L of magnesium (9.12 mg Ca and 1.01mg Mg per serving). Having a sports beverage that contains these important minerals is an added benefit to athletes, especially since there are no other commercial sports beverages that contain calcium or magnesium. Inadequate intake of calcium can contribute to inadequate amounts of calcium mineralization in the bones. A large portion of the United States, especially females over the age of 12 do not consume the recommended amounts of calcium. Not consuming enough calcium during bone mineralization can lead to osteoporosis. It is shown in elderly women that there is a correlation between current bone density and past calcium intake. Magnesium deficiencies due to an inadequate diet is relatively unheard of (Groff and Gropper, 2000).

We specifically estimated blood calcium and magnesium concentrations because they occur naturally in the whey permeate and we wanted to determine if there was any effect after consumption of the whey permeate beverage. The average blood concentrations of calcium and magnesium are presented in Table 11. The values for calcium (9.5-10.0 ppm) and magnesium (1.8-2.0 ppm) were very similar for the experimental whey permeate beverage, water Gatorade®, and beverage. The beverage contribution to blood calcium and magnesium is not apparent.

Table 11

Calcium and magnesium concentrations of blood before exercise, following exercise, and after consumption of the beverages

Mineral	Time of Blood Sampling	Gatorade		Water		Whey permeate beverage	
		Mean±SE	CV (%)	Mean±SE	CV (%)	Mean±SE	CV (%)
Ca	Before exercise	9.6±0.3	0.8	9.7±0.1	2.3	9.7±0.7	2.3
	Following exercise	9.8±0.2	0.6	9.9±0.1	2.7	10.0±0.5	1.7
	After beverage consumption	9.5±0.1	2.1	9.8±0.1	1.3	9.7±0.2	3.6
Mg	Before exercise	2.0±0.1	7.0	1.9±0.3	4.1	2.0±0.1	1.5
	Following exercise	1.9±0.1	6.9	1.8±0.3	3.8	1.9±0.1	1.0
	After beverage consumption	1.8±0.0	4.3	1.8±0.1	6.8	1.9±0.1	7.7

Blood sampling after 45 minutes of beverage consumption might not have provided enough time for the transport of calcium and magnesium. This is evident from the analysis of variance data (Tables12 and 13). The data in Table 12 showed no significant difference of blood calcium and magnesium levels before and after beverage consumption.

Table 12

Analysis of variance (ANOVA) of data of on calcium and magnesium concentrations of blood before exercise, following exercise, and after consumption of beverage

Mineral	Source of Variation	DF	Gatorade		Water		Whey permeate beverage	
			MSS	F	MSS	F	MSS	F
Ca	Time of blood sampling	2	0.076	2.73	0.073	1.59	0.100	0.691
	Error	9	0.028		0.050		0.144	
Mg	Time of blood sampling	2	0.033	2.33	0.031	1.46	0.008	0.788
	Error	9	0.014		0.021		0.010	

The data in Table 13 demonstrated no significant difference among three beverages (the experimental whey permeate beverage, water Gatorade®) in their contribution to blood calcium and magnesium levels. Further studies will be needed to evaluate additional benefits of sports drink based on whey permeate.

Table 13

Analysis of variance (ANOVA) of data of on calcium and magnesium of blood after consumption of Gatorade®, water and whey permeate beverage

Source of Variation	DF	Ca		Mg	
		MSS	F	MSS	F
Type of beverage	2	0.0694	1.15	0.0308	2.12
Error	9	0.0602		0.0145	

Chapter Five

Conclusions

The results of this study showed that electrolyte beverages such as the whey permeate based sports drink and Gatorade® acted similarly to water in whole body rehydration amongst selected athletes. The composition of the whey permeate based sports drink and Gatorade® were similar (6% carbohydrate, 110 mg sodium, and 30 mg of potassium). However the whey permeate beverage also had calcium and magnesium as natural components in the base.

The data did not indicate any influence of the experimental beverage, water and Gatorade® on mineral composition in the blood. A change in our experimental conditions (longer exercise period, longer resting period, controlled environment, more volunteers, standardized diet, beverage consumption during exercise) will be needed to prove the benefit of consuming a whey permeate beverage for restoring hydration status.

Recommendations for Further Study

Based on this study, the recommendations for further study are to:

- Study the effect of the whey permeate based sports beverage on blood mineral/electrolyte composition using a large population of endurance athletes (marathon runners, cyclists, etc.).
- Compare the effects of the whey permeate based sports beverage with commercial sports beverages allowing more resting time before the final blood draw.
- Explore the status of calcium and magnesium in whey permeate and their nutritional benefits to athletes.

- Compare analysis of urine to determine if urine production, concentration or volume is changed when different rehydration beverages are consumed.
- Initiate a survey to determine the athletes response and acceptability to the beverage being tested.

References

Cade, R., Spooner, G., Schlein, E., Pickering, M., & Dean R. (1972) Effect of fluid, electrolyte, and glucose replacement during exercise on performance, body temperature, rate of sweat loss, and compositional changes of extracellular fluid. *Journal of Sports Medicine, 12*, 150-156.

Carter, J. E., & Gisolfi, C.V. (1989). Fluid replacement during and after exercise in the heat. *Medicine and Science in Sports and Exercise, 21*, 532-539.

Coyle, E. F. (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *Journal of Applied Physiology, 61*, 165-172.

Choudhury, G. (2002) Personal communication. University of Wisconsin-Stout, Food and Nutrition Department. Menomonie Wisconsin.

Driskell, J. A., & Wolinsky, I. (1999). Macroelements, water, and electrolytes in sports nutrition. Boca Raton, FL: CRC Press LLC.

Gillies, M. T. (1974). Whey processing and utilization: Economic and technical aspects. Park Ridge, NY: Noyes Data Corporation.

Girsh, L. S. (1999). US Patent Number 5912040. June 15, 1999.

Gisolfi, C. V., Summers, R. D., Schedl, H. P., & Bleiler, T. L. (1995). Effect of sodium concentration in a carbohydrate-electrolyte solution on intestinal absorption. *Med. Science Sports Exercise 27* (10), 1414-1420.

Gonzalez-Alonso, Heaps, C. L., & Coyle, E. F. (1992). Rehydration after exercise with common beverages and water. *International Journal of Sports Medicine, 13*, 399-406.

Groff, J. L., & Gropper, S.D. (2000). Advanced nutrition and human metabolism third edition. Belmont, CA: Wadsworth.

Guyton A. C. (1991). *Textbook of Medical Physiology.* 8th ed. Philadelphia PA: WB Saunders Company, 150-151.

Hugh, W. (2000) Personal communication. Wisconsin Department of Agriculture, Trade and Consumer Protection. Madison, Wisconsin.

Maughan, R. J., Murray, R. (2001). Sports drinks, basic science and practical aspects. Boca Raton FL: CRC Press LLC.

Massicott, D., Peronnet, F., Brisson, G., Bakkouch, K., & Hillaire-Marcel C. (1989). Oxidation of a glucose polymer during exercise: Comparison with glucose and fructose. *Journal of Applied Physiology, 66*(1),179-183.

McArdle, W. D., Katch, F. I., & Katch. V.L. (1999). Sports and exercise nutrition. Baltimore, MD: Lippincott Williams and Wilkins.

Mitchell, J. B., (1988). Effects of carbohydrate ingestion on gastric emptying and exercise performance. *Medicine and Science in Sports and Exercise, 20,* 110-115.

Murray, R. (1987) The effects of consuming carbohydrate-electrolyte beverages on gastric emptying and fluid absorption during and following exercise. *Sports Medicine 4,* 323-351.

Murray, R., & Stofan, J. (2001). In sports drink: Basic science and practical aspects. New York, NY: CRC Press, 197-217.

Rosenbloom, C. A. (2000). Sports nutrition, a guide for the professional working with active people third edition. Chicago IL: The American Dietetic Association.

Schedl, H. P, Maughan, R. J., & Gisolfi, C. V. (1994). Intestinal absorption during rest and exercise: implication for formulating an oral rehydration solution (ORS). *Medicine and Science in Sports and Exercise. 26*, 267-280.

Shirreffs, S. M., Taylor, A. J., Leiper, K. B., & Maughan, R. J. (1996). Post exercise rehydration in man: effects of volume consumed and drink sodium content. *Medicine and Science in Sports and Exercise. 28*, 1260-1271.